



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 38/00, 39/395, 48/00, C07H 21/04, C07K 14/435, 14/705, 16/00, 16/18, 16/28, C12N 15/01, 15/11, 15/12, 15/63</b>		<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/53840</b>
			<b>(43) International Publication Date:</b> 3 December 1998 (03.12.98)
<b>(21) International Application Number:</b> PCT/US98/10791		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
<b>(22) International Filing Date:</b> 27 May 1998 (27.05.98)			
<b>(30) Priority Data:</b> 60/047,572 27 May 1997 (27.05.97) US			
<b>(71) Applicant (for all designated States except US):</b> SMITHKLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US).			
<b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> KIKLY, Kristine, Kay [US/US]; 499 Limerick Center Road, Linfield, PA 19468 (US). ERICKSON-MILLER, Connie, Lynn [US/US]; 608 Brainerd Place, Exton, PA 19341 (US).			
<b>(74) Agents:</b> ANDERSEN, Robert, L. et al.; Ratner & Prestia, Suite 301, One Westlakes, Berwyn, P.O. Box 980, Valley Forge, PA 19482-0980 (US).		<b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
<b>(54) Title:</b> SIALOADHESIN FAMILY 4 cDNA			
<b>(57) Abstract</b>  The SAF-4 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing SAF-4 polypeptides and polynucleotides in therapy, and diagnostic assays for such.			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Larvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakistan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

- 1 -

## **Sialoadhesin Family 4 cDNA**

### **Field of the Invention**

This invention relates to newly identified polypeptides and polynucleotides encoding such polypeptides, to their use in therapy and in identifying compounds which  
5 may be agonists, antagonists and /or inhibitors which are potentially useful in therapy, and to production of such polypeptides and polynucleotides.

### **Background of the Invention**

The drug discovery process is currently undergoing a fundamental revolution as it embraces 'functional genomics', that is, high throughput genome- or gene-based biology.  
10 This approach is rapidly superceding earlier approaches based on 'positional cloning'. A phenotype, that is a biological function or genetic disease, would be identified and this would then be tracked back to the responsible gene, based on its genetic map position.

Functional genomics relies heavily on the various tools of bioinformatics to identify gene sequences of potential interest from the many molecular biology databases now available.  
15 There is a continuing need to identify and characterize further genes and their related polypeptides/proteins, as targets for drug discovery.

### **Summary of the Invention**

The present invention relates to SAF-4, in particular SAF-4 polypeptides and SAF-4 polynucleotides, recombinant materials and methods for their production. In another aspect,  
20 the invention relates to methods for using such polypeptides and polynucleotides, including the treatment of cancer, inflammation, autoimmunity, allergy, asthma, rheumatoid arthritis, CNS inflammation, cerebellar degeneration, Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, head injury damage, and other neurological abnormalities, septic shock, sepsis, stroke, osteoporosis, osteoarthritis, ischemia reperfusion  
25 injury, cardiovascular disease, kidney disease, liver disease, ischemic injury, myocardial infarction, hypotension, hypertension, AIDS, myelodysplastic syndromes and other hematologic abnormalities, aplastic anemia, male pattern baldness, and bacterial, fungal, protozoan and viral infections, hereinafter referred to as "the Diseases", amongst others. In a further aspect, the invention relates to methods for identifying agonists and

- 2 -

antagonists/inhibitors using the materials provided by the invention, and treating conditions associated with SAF-4 imbalance with the identified compounds. In a still further aspect, the invention relates to diagnostic assays for detecting diseases associated with inappropriate SAF-4 activity or levels.

5

### Description of the Invention

In a first aspect, the present invention relates to SAF-4 polypeptides. Such peptides include isolated polypeptides comprising an amino acid sequence which has at least 70 % identity, preferably at least 80 % identity, more preferably at least 90 % identity, yet more preferably at least 95 % identity, most preferably at least 97-99 % identity, to that of SEQ  
10 ID NO:2 over the entire length of SEQ ID NO:2. Such polypeptides include those comprising the amino acid of SEQ ID NO:2.

Further peptides of the present invention include isolated polypeptides in which the amino acid sequence has at least 70 % identity, preferably at least 80 % identity, more preferably at least 90 % identity, yet more preferably at least 95 % identity, most  
15 preferably at least 97-99 % identity, to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2. Such polypeptides include the polypeptide of SEQ ID NO:2.

Further peptides of the present invention include isolated polypeptides encoded by a polynucleotide comprising the sequence contained in SEQ ID NO:1.

20

Polypeptides of the present invention are believed to be members of the Sialoadhesin family of polypeptides. They are therefore of interest because the sialoadhesin family of proteins, sialoadhesin, CD33, CD22 and myelin-associated glycoprotein (MAG), are utilized as cellular interaction molecules. They bind specific carbohydrates in a sialic acid-dependent manner on target cells. The extracellular domain is made up of various numbers of  
25 immunoglobulin-like domains of the V-like and C2-like subtypes and the intracellular portion has no known homology to any signalling motifs. Sialoadhesin expression is restricted to macrophages, it has 17 Ig-like domains and the specific recognition sequence on target cells is Neu5Aca2,3Galb13GalNAc. Known target cells are developing myeloid cells in the bone marrow and lymphocytes in the spleen and lymph node (Crocker, P. R., et al. EMBO J,

- 3 -

1994, 13:4490-4503). CD22 is expressed only on B cells and has a and b isoforms with 5 and 7 Ig-like domains, respectively. CD22 is known to bind T cells, B cells, monocytes, granulocytes and erythrocytes by recognizing Neu5Aca2,6Galb1,4Glc(NAc) in N-linked glycans (Crocker, P. R., et al. EMBO J, 1994, 13:4490-4503; Stamenkovic, I. and Seed, B. Nature, 1990, 345:74-77; Wilson, G. L., et al. J Exp Med, 1991, 173:137-146). Myelin-associated glycoprotein (MAG) is expressed by Schwann cells in the peripheral nervous system and oligodendrocytes in the central nervous system and is thought to participate in the cell adhesion to axons. MAG has two alternatively spliced variants, large MAG (L-MAG) and small MAG (S-MAG) which are expressed either during embryonic development or in the adult, respectively. The alternative splicing results in the expression of the same extracellular domains but distinct intracellular domains (Pedraza, L. et al., JCB, 1990, 111:2651-2661).

CD33 is most relevant to SAF-4 because they are the most closely related of all the family members. CD33 is normally expressed on the developing myelomonocytic lineage. It is absent on early stem cells but is present on colony-forming units for granulocytes, erythrocytes, monocytes, and megakaryocytes (CFU-GEMM) and progenitors of granulocytes and mononuclear phagocytes (CFU-GM). It is downregulated by mature granulocytes but retained by mature monocytes and macrophages (Andrews, R. G., et al., Blood, 1983, 62:124; Griffin, J. D., et al., Leuk Res 1984, 8:521). CD33 has two Ig-like domains and prefers to bind targets expressing NeuAca2,3Gal in N- and O-linked glycans. It maps to chromosome 19q13.1-13.3, closely linking it in the genome with MAG and CD22 (Freeman, S. D., et al., Blood, 1995, 85:2005-2012).

CD33 has also been found to be expressed on about 85 % of leukemic myeloblasts in patients with acute myelogenous leukemia (AML) and is frequently used to differentiate AML from acute lymphoblastic leukemia (ALL). Monoclonal antibodies to CD33 have been used therapeutically to purge residual myeloblasts from autologous bone marrow grafts ex vivo for the treatment of AML (Robertson, M. J., et al., Blood, 1992 79:2229-2236). More recently, humanized monoclonal antibodies to CD33 have undergone evaluation in vivo for the treatment of AML (Caron, P. C., et al., Blood, 1994, 83:1760-1768). These properties are hereinafter referred to as "SAF-4 activity" or "SAF-4 polypeptide activity" or "biological activity of SAF-4". Also included amongst these activities are antigenic and

- 4 -

immunogenic activities of said SAF-4 polypeptides, in particular the antigenic and immunogenic activities of the polypeptide of SEQ ID NO:2. Preferably, a polypeptide of the present invention exhibits at least one biological activity of SAF-4.

5 The polypeptides of the present invention may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

10 The present invention also includes include variants of the aforementioned polypeptides, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids  
15 are substituted, deleted, or added in any combination.

Polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

20 In a further aspect, the present invention relates to SAF-4 polynucleotides. Such polynucleotides include isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide which has at least 70 % identity, preferably at least 80 % identity, more preferably at least 90 % identity, yet more preferably at least 95 % identity, to the amino acid sequence of SEQ ID NO:2, over the entire length of SEQ ID NO:2. In this regard,  
25 polypeptides which have at least 97 % identity are highly preferred, whilst those with at least 98-99 % identity are more highly preferred, and those with at least 99 % identity are most highly preferred. Such polynucleotides include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding the polypeptide of SEQ ID NO:2.

- 5 -

Further polynucleotides of the present invention include isolated polynucleotides comprising a nucleotide sequence that has at least 70 % identity, preferably at least 80 % identity, more preferably at least 90 % identity, yet more preferably at least 95 % identity, to a nucleotide sequence encoding a polypeptide of SEQ ID NO:2, over the entire coding  
5 region. In this regard, polynucleotides which have at least 97 % identity are highly preferred, whilst those with at least 98-99 % identity are more highly preferred, and those with at least 99 % identity are most highly preferred.

Further polynucleotides of the present invention include isolated polynucleotides comprising a nucleotide sequence which has at least 70 % identity, preferably at least 80 %  
10 identity, more preferably at least 90 % identity, yet more preferably at least 95 % identity, to SEQ ID NO:1 over the entire length of SEQ ID NO:1. In this regard, polynucleotides which have at least 97 % identity are highly preferred, whilst those with at least 98-99 % identity are more highly preferred, and those with at least 99 % identity are most highly preferred. Such polynucleotides include a polynucleotide comprising the polynucleotide of  
15 SEQ ID NO:1 as well as the polynucleotide of SEQ ID NO:1.

The invention also provides polynucleotides which are complementary to all the above described polynucleotides.

The nucleotide sequence of SEQ ID NO:1 shows homology with CD33 (Simmons, D., and Seed, B., JI 141:2797-2800, 1988). The nucleotide sequence of SEQ ID NO:1 is a  
20 cDNA sequence and comprises a polypeptide encoding sequence (nucleotide 51 to 1970) encoding a polypeptide of 639 amino acids, the polypeptide of SEQ ID NO:2. The nucleotide sequence encoding the polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in SEQ ID NO:1 or it may be a sequence other than the one contained in SEQ ID NO:1, which, as a result of the redundancy  
25 (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2. The polypeptide of the SEQ ID NO:2 is structurally related to other proteins of the Sialoadhesin family, having homology and/or structural similarity with CD33 (Simmons, D., and Seed, B., JI 141:2797-2800, 1988).

Preferred polypeptides and polynucleotides of the present invention are expected to  
30 have, *inter alia*, similar biological functions/properties to their homologous polypeptides and

- 6 -

polynucleotides. Furthermore, preferred polypeptides and polynucleotides of the present invention have at least one SAF-4 activity.

The present invention also relates to partial or other polynucleotide and polypeptide sequences which were first identified prior to the determination of the corresponding full  
5 length sequences of SEQ ID NO:1 and SEQ ID NO:2.

Accordingly, in a further aspect, the present invention provides for an isolated polynucleotide comprising:

- (a) a nucleotide sequence which has at least 70 % identity, preferably at least 80 % identity, more preferably at least 90 % identity, yet more preferably at least 95 % identity,  
10 even more preferably at least 97-99 % identity to SEQ ID NO:3 over the entire length of SEQ ID NO:3;
  - (b) a nucleotide sequence which has at least 70 % identity, preferably at least 80 % identity, more preferably at least 90 % identity, yet more preferably at least 95 % identity, even more preferably at least 97-99 % identity, to SEQ ID NO:3 over the entire length of  
15 SEQ ID NO:3;
  - (c) the polynucleotide of SEQ ID NO:3; or
  - (d) a nucleotide sequence encoding a polypeptide which has at least 70 % identity, preferably at least 80 % identity, more preferably at least 90 % identity, yet more preferably at least 95 % identity, even more preferably at least 97-99 % identity, to the amino acid sequence of  
20 SEQ ID NO:4, over the entire length of SEQ ID NO:4;
- as well as the polynucleotide of SEQ ID NO:3.

The present invention further provides for a polypeptide which:

- (a) comprises an amino acid sequence which has at least 70 % identity, preferably at least 80 % identity, more preferably at least 90 % identity, yet more preferably at least 95 %  
25 identity, most preferably at least 97-99 % identity, to that of SEQ ID NO:4 over the entire length of SEQ ID NO:4;
- (b) has an amino acid sequence which is at least 70 % identity, preferably at least 80 % identity, more preferably at least 90 % identity, yet more preferably at least 95 % identity,



- 7 -

most preferably at least 97-99% identity, to the amino acid sequence of SEQ ID NO:4 over the entire length of SEQ ID NO:4;

(c) comprises the amino acid of SEQ ID NO:4; and

(d) is the polypeptide of SEQ ID NO:4;

5 as well as polypeptides encoded by a polynucleotide comprising the sequence contained in SEQ ID NO:3.

The nucleotide sequence of SEQ ID NO:3 and the peptide sequence encoded thereby are derived from EST (Expressed Sequence Tag) sequences. It is recognized by those skilled in the art that there will inevitably be some nucleotide sequence reading  
10 errors in EST sequences (see Adams, M.D. *et al*, Nature 377 (supp) 3, 1995). Accordingly, the nucleotide sequence of SEQ ID NO:3 and the peptide sequence encoded therefrom are therefore subject to the same inherent limitations in sequence accuracy. Furthermore, the peptide sequence encoded by SEQ ID NO:3 comprises a region of identity or close homology and/or close structural similarity (for example a conservative  
15 amino acid difference) with the closest homologous or structurally similar protein.

Polynucleotides of the present invention may be obtained, using standard cloning and screening techniques, from a cDNA library derived from mRNA in cells of human primary dendritic cells, using the expressed sequence tag (EST) analysis (Adams, M.D., *et al*. *Science* (1991) 252:1651-1656; Adams, M.D. *et al*., *Nature*, (1992) 355:632-634;  
20 Adams, M.D., *et al*., *Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

When polynucleotides of the present invention are used for the recombinant production of polypeptides of the present invention, the polynucleotide may include the  
25 coding sequence for the mature polypeptide, by itself; or the coding sequence for the mature polypeptide in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention,

- 8 -

the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further embodiments of the present invention include polynucleotides encoding polypeptide variants which comprise the amino acid sequence of SEQ ID NO:2 and in which several, for instance from 5 to 10, 1 to 5, 1 to 3, 1 to 2 or 1, amino acid residues are substituted, deleted or added, in any combination.

Polynucleotides which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1, may be used as hybridization probes for cDNA and genomic DNA or as primers for a nucleic acid amplification (PCR) reaction, to isolate full-length cDNAs and genomic clones encoding polypeptides of the present invention and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human) that have a high sequence similarity to SEQ ID NO:1. Typically these nucleotide sequences are 70 % identical, preferably 80 % identical, more preferably 90 % identical, most preferably 95 % identical to that of the referent. The probes or primers will generally comprise at least 15 nucleotides, preferably, at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will have between 30 and 50 nucleotides.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than human, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO: 1 or a fragment thereof; and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to the skilled artisan. Preferred stringent hybridization conditions include overnight incubation at 42°C in a solution comprising: 50 % formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA; followed by washing the filters in 0.1x SSC at about 65°C.

- 9 -

Thus the present invention also includes polynucleotides obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:1 or a fragment thereof.

The skilled artisan will appreciate that, in many cases, an isolated cDNA sequence will be incomplete, in that the region coding for the polypeptide is cut short at the 5' end of the cDNA. This is a consequence of reverse transcriptase, an enzyme with inherently low 'processivity' (a measure of the ability of the enzyme to remain attached to the template during the polymerization reaction), failing to complete a DNA copy of the mRNA template during 1st strand cDNA synthesis.

There are several methods available and well known to those skilled in the art to obtain full-length cDNAs, or extend short cDNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman et al., PNAS USA 85, 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon™ technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the 'missing' 5' end of the cDNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using 'nested' primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the known gene sequence). The products of this reaction can then be analysed by DNA sequencing and a full-length cDNA constructed either by joining the product directly to the existing cDNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

Recombinant polypeptides of the present invention may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems.

Accordingly, in a further aspect, the present invention relates to expression systems which comprise a polynucleotide or polynucleotides of the present invention, to host cells which are

- 10 -

genetically engineered with such expression systems and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

5 For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., Basic Methods in Molecular Biology (1986) and Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor  
10 Laboratory Press, Cold Spring Harbor, N.Y. (1989). Preferred such methods include, for instance, calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as  
15 *streptococci*, *staphylococci*, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used, for instance, chromosomal,  
20 episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and  
25 bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector which is able to maintain, propagate or express a polynucleotide to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for  
30 example, those set forth in Sambrook et al., *MOLECULAR CLONING, A LABORATORY*

- 11 -

*MANUAL (supra)*. Appropriate secretion signals may be incorporated into the desired polypeptide to allow secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals.

5           If a polypeptide of the present invention is to be expressed for use in screening assays, it is generally preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If the polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide. If produced intracellularly, the cells must first be lysed before the  
10   polypeptide is recovered.

          Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography,  
15   hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

          This invention also relates to the use of polynucleotides of the present invention as  
20   diagnostic reagents. Detection of a mutated form of the gene characterized by the polynucleotide of SEQ ID NO:1 which is associated with a dysfunction will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, or susceptibility to a disease, which results from under-expression, over-expression or altered expression of the gene. Individuals carrying mutations in the gene may be detected at the DNA level by a  
25   variety of techniques.

          Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions  
30   and insertions can be detected by a change in size of the amplified product in comparison to

- 12 -

the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled SAF-4 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing (see, e.g., Myers *et al.*, *Science* (1985) 230:1242). Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (see Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401). In another embodiment, an array of oligonucleotide probes comprising SAF-4 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see for example: M. Chee *et al.*, *Science*, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to the Diseases through detection of mutation in the SAF-4 gene by the methods described. In addition, such diseases may be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of polypeptide or mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, nucleic acid amplification, for instance PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as a polypeptide of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays and flow cytometric analysis.

Thus in another aspect, the present invention relates to a diagnostic kit which comprises:

(a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof ;

- 13 -

- (b) a nucleotide sequence complementary to that of (a);
- (c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO:2 or a fragment thereof; or
- (d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of  
5 SEQ ID NO:2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a disease, particularly cancer, inflammation, autoimmunity, allergy, asthma, rheumatoid arthritis, CNS inflammation, cerebellar degeneration, Alzheimer's disease, Parkinson's  
10 disease, multiple sclerosis, amyotrophic lateral sclerosis, head injury damage, and other neurological abnormalities, septic shock, sepsis, stroke, osteoporosis, osteoarthritis, ischemia reperfusion injury, cardiovascular disease, kidney disease, liver disease, ischemic injury, myocardial infarction, hypotension, hypertension, AIDS, myelodysplastic syndromes and other hematologic abnormalities, aplastic anemia, male pattern baldness, and bacterial, fungal,  
15 protozoan and viral infections, amongst others.

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to, and can hybridize with, a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those  
20 sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, V. McKusick, Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the  
25 same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the

- 14 -

affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them, can also be used as immunogens to produce antibodies immunospecific for polypeptides of the present invention. The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against polypeptides of the present invention may be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a non-human animal, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies, such as those described in U.S. Patent No. 4,946,778, can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms, including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against polypeptides of the present invention may also be employed to treat the Diseases, amongst others. Antibodies against SAF-4 polypeptides may also be employed to subcharacterize cell populations during hematopoietic development, as a diagnostic marker to distinguish between different forms of cancer, to purge bone marrow ex vivo of cancer cells expressing SAF-4, as a tool to aid in the ex vivo expansion (proliferation and/or differentiation) of hematopoietic progenitor cells expressing SAF-4, as a stimulus in vivo for stem cell mobilization into the periphery, and as an in vivo chemoprotective agent.



- 15 -

In a further aspect, the present invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. In another approach, soluble forms of SAF-4 polypeptides still capable of binding the ligand in competition with endogenous SAF-4 may be administered. Typical embodiments of such competitors comprise fragments of the SAF-4 polypeptide. One example is using the extracellular domain of SAF-4 fused to a human immunoglobulin Fc region which can then be employed to treat cancer, inflammation, autoimmunity and allergy, among others. SAF-4/Fc polypeptides may also be employed to purge bone marrow ex vivo of cancer cells expressing SAF-4 ligands, as a tool to aid in the ex vivo expansion (proliferation and/or differentiation) of hematopoietic progenitor cells expressing SAF-4 ligands, as a stimulus in vivo for stem cell mobilization into the periphery, and as an in vivo chemoprotective agent. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with a polypeptide of the present invention, adequate to produce antibody and/or T cell immune response to protect said animal from the Diseases hereinbefore mentioned, amongst others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering a polypeptide of the present invention *via* a vector directing expression of the polynucleotide and coding for the polypeptide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

- 16 -

A further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a polypeptide of the present invention wherein the composition comprises a polypeptide or polynucleotide of the present invention. The vaccine formulation may further comprise a suitable carrier. Since a polypeptide may be broken down in the stomach, it is preferably administered parenterally (for instance, subcutaneous, intramuscular, intravenous, or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Polypeptides of the present invention are responsible for many biological functions, including many disease states, in particular the Diseases hereinbefore mentioned. It is therefore desirable to devise screening methods to identify compounds which stimulate or which inhibit the function of the polypeptide. Accordingly, in a further aspect, the present invention provides for a method of screening compounds to identify those which stimulate or which inhibit the function of the polypeptide. In general, agonists or antagonists may be employed for therapeutic and prophylactic purposes for such Diseases as hereinbefore mentioned. Compounds may be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. Such agonists, antagonists or inhibitors so-identified may be natural or modified substrates, ligands, receptors, enzymes, etc., as the case may be, of the polypeptide; or may be structural or functional mimetics thereof (see Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991)).

- 17 -

The screening method may simply measure the binding of a candidate compound to the polypeptide, or to cells or membranes bearing the polypeptide, or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound. Alternatively, the screening method may involve competition with a labeled competitor.

5 Further, these screening methods may test whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells bearing the polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Constitutively active polypeptides  
10 may be employed in screening methods for inverse agonists or inhibitors, in the absence of an agonist or inhibitor, by testing whether the candidate compound results in inhibition of activation of the polypeptide. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide of the present invention, to form a mixture, measuring SAF-4 activity in the mixture, and  
15 comparing the SAF-4 activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and SAF-4 polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists for the polypeptide of the present invention (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

20 The polynucleotides, polypeptides and antibodies to the polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the  
25 art. This can be used to discover agents which may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The polypeptide may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. These include, but  
30 are not limited to, ligand binding and crosslinking assays in which the polypeptide is

- 18 -

labeled with a radioactive isotope (for instance,  $^{125}\text{I}$ ), chemically modified (for instance, biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (cells, cell membranes, cell supernatants, tissue extracts, bodily fluids). Other methods include biophysical techniques such as  
5 surface plasmon resonance and spectroscopy. These screening methods may also be used to identify agonists and antagonists of the polypeptide which compete with the binding of the polypeptide to its receptors, if any. Standard methods for conducting such assays are well understood in the art.

Examples of potential polypeptide antagonists include antibodies or, in some cases,  
10 oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, enzymes, etc., as the case may be, of the polypeptide, e.g., a fragment of the ligands, substrates, receptors, enzymes, etc.; or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

15 Thus, in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for polypeptides of the present invention; or compounds which decrease or enhance the production of such polypeptides, which comprises:

- (a) a polypeptide of the present invention;
- 20 (b) a recombinant cell expressing a polypeptide of the present invention;
- (c) a cell membrane expressing a polypeptide of the present invention; or
- (d) antibody to a polypeptide of the present invention;

which polypeptide is preferably that of SEQ ID NO:2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a  
25 substantial component.

It will be readily appreciated by the skilled artisan that a polypeptide of the present invention may also be used in a method for the structure-based design of an agonist, antagonist or inhibitor of the polypeptide, by:

- 19 -

- (a) determining in the first instance the three-dimensional structure of the polypeptide;
- (b) deducing the three-dimensional structure for the likely reactive or binding site(s) of an agonist, antagonist or inhibitor;
- (c) synthesizing candidate compounds that are predicted to bind to or react with the
- 5 deduced binding or reactive site; and
- (d) testing whether the candidate compounds are indeed agonists, antagonists or inhibitors.

It will be further appreciated that this will normally be an interactive process.

In a further aspect, the present invention provides methods of treating abnormal

10 conditions such as, for instance, cancer, inflammation, autoimmunity, allergy, asthma, rheumatoid arthritis, CNS inflammation, cerebellar degeneration, Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, head injury damage, and other neurological abnormalities, septic shock, sepsis, stroke, osteoporosis, osteoarthritis, ischemia reperfusion injury, cardiovascular disease, kidney disease, liver disease, ischemic

15 injury, myocardial infarction, hypotension, hypertension, AIDS, myelodysplastic syndromes and other hematologic abnormalities, aplastic anemia, male pattern baldness, and bacterial, fungal, protozoan and viral infections, related to either an excess of, or an under-expression of, SAF-4 polypeptide activity.

If the activity of the polypeptide is in excess, several approaches are available. One

20 approach comprises administering to a subject in need thereof an inhibitor compound (antagonist) as hereinabove described, optionally in combination with a pharmaceutically acceptable carrier, in an amount effective to inhibit the function of the polypeptide, such as, for example, by blocking the binding of ligands, substrates, receptors, enzymes, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another

25 approach, soluble forms of the polypeptides still capable of binding the ligand, substrate, enzymes, receptors, etc. in competition with endogenous polypeptide may be administered. Typical examples of such competitors include fragments of the SAF-4 polypeptide.

- 20 -

In still another approach, expression of the gene encoding endogenous SAF-4 polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered (see, for example, O'Connor, *J Neurochem* (1991) 56:560 in

5 Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Alternatively, oligonucleotides which form triple helices with the gene can be supplied (see, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360). These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

10 For treating abnormal conditions related to an under-expression of SAF-4 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates a polypeptide of the present invention, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy

15 may be employed to effect the endogenous production of SAF-4 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such

20 that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For an overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers

25 Ltd (1996). Another approach is to administer a therapeutic amount of a polypeptide of the present invention in combination with a suitable pharmaceutical carrier.

In a further aspect, the present invention provides for pharmaceutical compositions comprising a therapeutically effective amount of a polypeptide, such as the soluble form of a polypeptide of the present invention, agonist/antagonist peptide or small molecule compound,

30 in combination with a pharmaceutically acceptable carrier or excipient. Such carriers include,

- 21 -

but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The composition will be adapted to the route of administration, for instance by a systemic or an oral route. Preferred forms of systemic administration include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if a polypeptide or other compounds of the present invention can be formulated in an enteric or an encapsulated formulation, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels, and the like.

The dosage range required depends on the choice of peptide or other compounds of the present invention, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100  $\mu\text{g/kg}$  of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

- 22 -

Polynucleotide and polypeptide sequences form a valuable information resource with which to identify further sequences of similar homology. This is most easily facilitated by storing the sequence in a computer readable medium and then using the stored data to search a sequence database using well known searching tools, such as GCC. Accordingly, in a further aspect, the present invention provides for a computer readable medium having stored thereon a polynucleotide comprising the sequence of SEQ ID NO:1 and/or a polypeptide sequence encoded thereby.

The following definitions are provided to facilitate understanding of certain terms used frequently hereinbefore.

10       “Antibodies” as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

      “Isolated” means altered “by the hand of man” from the natural state. If an “isolated” composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not “isolated,” but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is “isolated”, as the term is employed herein.

      “Polynucleotide” generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. “Polynucleotides” include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, “polynucleotide” refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term “polynucleotide” also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. “Modified” bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus,



- 23 -

“polynucleotide” embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. “Polynucleotide” also embraces relatively short polynucleotides, often referred to as oligonucleotides.

- 5           “Polypeptide” refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. “Polypeptide” refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids.
- 10          “Polypeptides” include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications may occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-
- 15          chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present to the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result
- 20          from post-translation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond
- 25          formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation,
- 30          and ubiquitination (see, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York,

- 24 -

1993; Wold, F., Post-translational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and  
5 Rattan *et al.*, "Protein Synthesis: Post-translational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62).

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide.  
10 Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide.  
15 Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or  
20 polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as the case may be, as determined by  
25 comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer*  
30

- 25 -

*Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

Parameters for polypeptide sequence comparison include the following:

1) Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci. USA.* 89:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Parameters for polynucleotide comparison include the following:

1) Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

- 26 -

**Gap Length Penalty: 3**

Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

5 A preferred meaning for "identity" for polynucleotides and polypeptides, as the case may be, are provided in (1) and (2) below.

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the reference sequence of SEQ ID NO:1, wherein said polynucleotide sequence may be identical to the reference sequence of SEQ ID NO: 1 or may include up to  
10 a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the  
15 nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$20 \quad n_n \leq x_n - (x_n \bullet y),$$

wherein  $n_n$  is the number of nucleotide alterations,  $x_n$  is the total number of nucleotides in SEQ ID NO:1,  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and  $\bullet$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_n$  and  $y$  is rounded  
25 down to the nearest integer prior to subtracting it from  $x_n$ . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

- 27 -

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is it may be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one nucleic acid deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleic acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleic acid alterations for a given percent identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_n \leq x_n - (x_n \bullet y),$$

wherein  $n_n$  is the number of amino acid alterations,  $x_n$  is the total number of amino acids in SEQ ID NO:2,  $y$  is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc.,  $\bullet$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_n$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_n$ .

(2) Polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, wherein said polypeptide sequence may be identical to the reference sequence of SEQ ID NO: 2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid

- 28 -

alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \bullet y),$$

5 wherein  $n_a$  is the number of amino acid alterations,  $x_a$  is the total number of amino acids in SEQ ID NO:2,  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and  $\bullet$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_a$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_a$ .

10 By way of example, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is it may be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one amino acid deletion,  
 15 substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for  
 20 a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \bullet y),$$

wherein  $n_a$  is the number of amino acid alterations,  $x_a$  is the total number of amino acids  
 25 in SEQ ID NO:2,  $y$  is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and  $\bullet$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_a$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_a$ .

- 29 -

"Fusion protein" refers to a protein encoded by two, often unrelated, fused genes or fragments thereof. In one example, EP-A-0 464 discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, employing an immunoglobulin Fc region  
5 as a part of a fusion protein is advantageous for use in therapy and diagnosis resulting in, for example, improved pharmacokinetic properties [see, e.g., EP-A 0232 262]. On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified.

All publications, including but not limited to patents and patent applications, cited  
10 in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

- 30 -

## SEQUENCE INFORMATION

## SEQ ID NO:1

TCCGAGAGAACAGGCCTGTCTCAGGCAGGCCCTGCGCCTCCTATGCGGAGATGCTACTGCCACTGCTGCTGTCC  
TCGCTGCTGGGCGGGTCCCAGGCTATGGATGGGAGATTCTGGATACGAGTGCAGGAGTCAGTGATGGTGCCGGA  
5 GGGCCTGTGCATCTCTGTGCCCTGCTCTTTCTCCTACCCCCGACAAGACTGGACAGGGTCTACCCCAGCTTATG  
GCTACTGGTTCAAAGCAGTGACTGAGACAACCAAGGGTGCTCCTGTGGCCACAAACCACCAGAGTCGAGAGGTG  
GAAATGAGCACCCGGGGCCGATTCCAGCTCACTGGGGATCCCGCCAAGGGGAAGTGTCTCCTTGGTGATCAGAGA  
CGCGCAGATGCAGGATGAGTCACAGTACTTCTTTCTGGGTGGAGAGAGGAAGCTATGTGAGATATAATTTTCATGA  
ACGATGGGTTCTTTCTAAAAGTAACAGTGCTCAGCTTCACGCCCAGACCCCAGGACCACAACACCGACCTCACC  
10 TGCCATGTGGACTTCTCCAGAAAGGGTGTGAGCGCACAGAGGACCGTCCGACTCCGTGTGGCCTATGCCCCCAG  
AGACCTTGTATCAGCATTTTACGTGACAACACGCCAGCCCTGGAGCCCCAGCCCCAGGGAAATGTCCCATAACC  
TGGAAGCCCCAAAAGGCCAGTTCTTGC GGCTCCTCTGTGCTGCTGACAGCCAGCCCCCTGCCACACTGAGCTGG  
GTCCTGCAGAACAGAGTCCTCTCCTCGTCCCATCCCTGGGGCCCTAGACCCCTGGGGCTGGAGCTGCCCCGGGGT  
GAAGGCTGGGGATTCAAGGCGCTACACCTGCCGAGCGGAGAACAGGCTTGGCTCCAGCAGCGAGCCCTGGACC  
15 TCTCTGTGCAGTATCCTCCAGAGAACCTGAGAGTGATGGTTTCCCAAGCAAACAGGACAGTCCTGGAAAACCTT  
GGGAACGGCACGTCTCTCCAGTACTGGAGGGCCAAAGCCTGTGCCCTGGTCTGTGTACACACAGCAGCCCCC  
AGCCAGGCTGAGCTGGACCCAGAGGGGACAGGTTCTGAGCCCTCCAGCCCTCAGACCCCGGGTCTTGAGC  
TGCCCTCGGGTTCAAGTGGAGCACGAAGGAGAGTTACCTGCCACGCTCGGCACCCACTGGGCTCCAGCACGTC  
TCTCTCAGCCTCTCCGTGCACTACTCCCCGAAGCTGCTGGGGCCCTCCTGCTCCTGGGAGGCTGAGGGTCTGCA  
20 CTGCAGCTGCTCCTCCAGGCCAGCCCGGCCCCCTCTCTGCGCTGGTGGCTTGGGGAGGAGCTGCTGGAGGGGA  
ACAGCAGCCAGGACTCCTTCGAGGTACCCCCAGCTCAGCCGGGCCCTGGGCCAACAGCTCCCTGAGCTCCAT  
GGAGGGCTCAGCTCCGGCCTCAGGCTCCGCTGTGAGGCTTGAACGTCCATGGGGCCAGAGTGGATCCATCCT  
GCAGCTGCCAGATAAGAAGGACTCATCTCAACGGCATTCTCCAACGGAGCGTTTCTGGGAATCGGCATCACGG  
CTCTTCTTTTCTCTGCTGCTGGCCCTGATCATCATGAAGATTCTACCGAAGAGACGGACTCAGACAGAAACCCG  
25 AGGCCCAGGTTCTCCGGCACAGCACGATCCTGGATTACATCAATGTGGTCCCGACGGCTGGCCCCCTGGCTCA  
GAAGCGGAATCAGAAAGCCACACCAAACAGTCCTCGGACCCCTCTTCCACCAGGTGCTCCCTCCCCAGAATCAA  
AGAAGAACCAGAAAAGCAGTATCAGTTGCCCAGTTTCCAGAACCCAAATCATCCACTCAAGCCCCAGAATCC  
CAGGAGAGCCAAGAGGAGCTCATTATGCCACGCTCAACTTCCAGGCGTCAGACCCAGGCCTGAGGCCCGGAT  
GCCCAAGGGCACCCAGGCGGATTATGCAGAAGTCAAGTTCCAATGAGGGTCTCTTAGGCTTTAGGACTGGGACT  
30 TCGGCTAGGGAGGAAGGTAGAGTAAGAGGTTGAAGATAACAGAGTGCAAAGTTTCTTCTCTCCCTCTCTCTCT  
CTCTTTCTCTCTCTCTCTCTCTTTCTCTCTCTTTTAAAAAACATCTGGCCAGGCAGCACAGTGGCTCACGCCGT  
AATCCCAGCACTTTGGGAGGTTGAGGTGGGCAGATCGCCTGAGGTGGGAGTTGAGACCAGCCTGGCCAACCTT  
GGTGAAACCCGCTCTCTACTAAAAATACAAAAATTAGCTGGGCATGGTGGCAGGCGCCTGTAATCCTACTACT  
TGGAAGCTGAGGCAGGAGAATCACTTGAACCTGGGAGACGGAGGTTGCAGTGAGCCAAGATCACACCATTGCA  
35 CGCCAGCCTGGGCAACAAAGCGAGACTCCATCTCAAAAAAAAAATCCTCCAAATGGGTTGGGTGTCTGTAATCC  
CAGCACTTTGGGAGGCTAAGGTGGGTGGATTGCTTGAGCCAGGAGTTGAGAGACCAGCCTGGGCAACATGGTGA  
AACCCCATCTCTACAAAAATACAAAAATAGCTGGGCTTGGTGGTGTGTGCTGTAAAGTCCCCAGCTGTGAGA



- 31 -

CATTTAAACCAGAGCAACTCCATCXTGGAATAGGAGGCTGAATAAAATGAGGCTGAGACCTXACTGGGCTGCAT  
TCTCCAGACAGTGGAGGCATTCTAAGTCACAGGATGAGACAGGAGGTCCCGTACAAGATACAGGTCATAAAGAC  
TTTGCTGATAAAACAGATTGCAGTAAAGAAGCCAACCAATCCCACCAAACCAAGTTGGCCACGAGAGTGACC  
TCTGGTCGTCTCACTGCTACACTCCTGACAGCACCATGACAGTTTACAAATGCCATGGCAACATCAGGAAGTT  
5 ACCCGATATGTCCCAAAGGGGGAGGAATGAATAATCCACCCCTTGTTTAGCAAATAAGCAAGAAATAACCATA  
AAAGTGGGCAACCAGCAGCTCTAGGCGCTGCTCTTGTCCTATGGAGTAGCCTTCTTTTCTTTAACTTTCTT  
GATAAACTTGCTTTTACCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

10 **SEQ ID NO:2**

MLLPLLLSSLLGGSQAMDGRFWIRVQESVMVPEGLCISVPCSFSPYPRQDWTGSTPAYGYWFKAVTETTKGAPVA  
TNHQSREVEVEMSTRGRFQLTGDPKAGNCSLVIRDAQMDESQYFFRVERGSYVRYNFMNDGFFLKVTVLSFTPRP  
QDHNTDLTCHVDFSRKGVSAQRTVRLRVAYAPRDLVISISRDNTPALEPQPQGNVPYLEAQKGQFLRLCAADS  
QPPATLSWVLQNRVLSSSHWPWPRLGLELPGVKAGDSGRYTCAENRLGSQQRALDLSVQYPENLRVMVSQA  
15 NRTVLENLNGTSLPVLEGQSLCLVCVTHSSPPARLSWTQRGQVLSPSQSPDPGVLELPRVQVEHEGEFTCHAR  
HPLGSQHVSLSLSVHYSPLKLLGPSCSWEAEGHLCSCSSQASPAPSLRWLGEELLEGNSSQDSFEVTPSSAGPW  
ANSSLSLHGGLSSGLRLRCEAWNVAQSGSILQLPDKKGLISTAFSNGAFLGIGITALLLFLCLALIIMKILPK  
RRTQTETPRPRFSRHSTILDYINVVPTAGPLAQKRNQKATPNSPRTPLPPGAPSPESKKNQKKQYQLPSFPEPK  
SSTQAPESQESQEELHYATLNFPGVRPRPEARMPKGTQADYAEVKFQ.

20

**SEQ ID NO:3**

TCCGAGAGAACAGGCCTGTCTCAGGCAGGCCCTGCGCCTCCTATGCGGAGATGCTACTGCCACTGCTGCTGTCC  
TCGCTGCTGGGCGGGTCCCAGGCTATGGATGGGAGATTCTGGATACGAGTGCAGGAGTCAGTGATGGTGCCGA  
25 GGGCCTGTGCATCTCTGTGCCCTGCTCTTTCTCTACCCCCGACAAGACTGGACAGGGTCTACCCAGCTTATG  
GCTACTGGTTCAAAGCAGTGACTGAGACAACCAAGGGTGCTCCTGTGGCCACAAACCACCAGAGTCGAGAGGTG  
GAAATGAGCACCCGGGGCCGATTCCAGCTCACTGGGGATCCCGCCAAGGGGAAGTGTCTCTTGGTGATCAGAGA  
CGCGCAGATGCAGGATGAGTCACAGTACTTCTTTGCGGTGGAGAGAGGAAGCTATGTGAGATATAATTTTCATGA  
ACGATGGGTCTTTCTAAAAGTAACAGTGCTCAGCTTCACGCCCAGACCCAGGACCACAACACCGACCTCACC  
30 TGCCATGTGGACTTCTCCAGAAAGGGTGTGAGCGCACAGAGGACCGTCCGACTCCGTGTGGCCTATGCCCCCAG  
AGACCTTGTTATCAGCATTTTCAGTGACAACACGCCAGCCCTGGAGCCCCAGCCCCAGGGAAATGTCCCATACC  
TGGAAGCCCCAAAAGGCCAGTTCTCTGCGGCTCCTCTGTGCTGCTGACAGCCAGCCCCCTGCCACACTGAGCTGG  
GTCTCTGCAGAACAGAGTCTCTCTCGTCCCATCCCTGGGGCCCTAGACCCCTGGGGCTGGAGCTGCCCGGGGT  
GAAGGCTGGGGATTTCAGGGCGCTACACCTGCCGAGCGGAGAACAGGCTTGGCTCCAGCAGCGAGCCCTGGACC  
35 TCTCTGTGCAGTATCCTCCAGAGAACCTGAGAGTGATGGTTTCCCAAGCAAACAGGACAGTCTTGAAAACCTT  
GGGAACGGCACGTCTCTCCAGTACTGGAGGGCCAAAGCCTGTGCTGGTCTGTGTACACACAGCAGCCCCC

- 32 -

AGCCAGGCTGAGCTGGACCCAGAGGGGACAGGTTCTGAGCCCCCTCCAGCCCTCAGACCCCGGGGTCTTGAGAGC  
TGCCTCGGGTTCAAGTGGAGCACGAAGGAGAGTTACCTGCCACGCTCGGCACCCACTGGGCTCCAGCACGTC  
TCTCTCAGCCTCTCCGTGCACTACTCCCCGAAGCTGCTGGGCCCTCCTGCTCCTGGGAGGCTGAGGGTCTGCA  
CTGCAGCTGCTCCTCCAGGCCAGCCCGCCCCCTCTCTGCGCTGGTGGCTTGGGGAGGAGCTGCTGGAGGGGA  
5 ACAGCAGCCAGGACTCCTTCGAGGTACCCCCAGCTCAGCCGGGCCCTGGGCCAACAGCTCCCTGAGCCTCCAT  
GGAGGGCTCAGCTCCGGCCTCAGGCTCCGCTGTGAGGCCTGGAACGTCCATGGGGCCAGAGTGGATCCATCCT  
GCAGCTGCCAGATAAGAAGGGACTCATCTCAACGGCATTTCTCCAACGGAGCGTTTCTGGGAATCGGCATCACGG  
CTCTTCTTTTCTCTGCTGGCCCTGATCATCATGAAGATTCTACCGAAGAGACGGACTCAGACAGAAACCCCG  
AGGCCCAGGTTCTCCCGGCACAGCACGATCCTGGATTACATCAATGTGGTCCCAGCGCTGGCCCCCTGGCTCA  
10 GAAGCGGAATCAGAAAGCCACACCAAACAGTCTCGGACCCCTCTTCCACCAGGTGCTCCCTCCCCAGAATCAA  
AGAAGAACCAGAAAAAGCAGTATCAGTTGCCCAGTTTCCAGAACCCAAATCATCCACTCAAGCCCCAGAATCC  
CAGGAGAGCCAAGAGGAGCTCCATTATGCCACGCTCAACTTCCAGGCGTCAGACCCAGGCCTGAGGCCCGGAT  
GCCCAAGGGCACCCAGGCGGATTATGCAGAAAGTCAAGTTCCAATGAGGGTCTCTTAGGCTTTAGGACTGGGACT  
TCGGCTAGGGAGGAAGGTAGAGTAAGAGGTTGAAGATAACAGAGTGCAAAGTTTCTTCTCTCCCTCTCTCTCT  
15 CTCTTTCTCTCTCTCTCTCTCTTTCTCTCTCTTTAAAAAACATCTGGCCAGGCAGCACAGTGGCTCACGCCTGT  
AATCCCAGCACTTTGGGAGGTTGAGGTGGGCAGATCGCCTGAGGTCGGGAGTTGAGACCAGCCTGGCCAACTTT  
GGTGAAACCCCGTCTCTACTAAAAATACAAAAATTAGCTGGGCATGGTGGCAGGCGCCTGTAATCCTACTACT  
TGGAAGCTGAGGCAGGAGAATCACTTGAACCTGGGAGACGGAGGTTGCAGTGAGCCAAGATCACACCATTGCA  
CGCCAGCCTGGGCAACAAAGCGAGACTCCATCTCAAAAAAAAAAATCCTCAAATGGGTTGGGTGTCTGTAATCC  
20 CAGCACTTTGGGAGGCTAAGGTGGGTGGATTGCTTGAGCCAGGAGTTTCGAGACCAGCCTGGGCAACATGGTGA  
AACCCCATCTCTACAAAAATACAAAAATAGCTGGGCTTGGTGGTGTGTGCCTGTAAGTCCCAGCTGTCAGA  
CATTTAAACCAGAGCAACTCCATCXTGGAATAGGAGGCTGAATAAAATGAGGCTGAGACCTXACTGGGCTGCAT  
TCTCCAGACAGTGGAGGCATTCTAAGTCACAGGATGAGACAGGAGGTCCCGTACAAGATACAGGTCATAAAGAC  
TTTGCTGATAAAACAGATTGCAGTAAAGAAGCCAACCAAATCCCACCAAACCAAGTTGGCCACGAGAGTGACC  
25 TCTGGTCGTCCTCACTGCTACACTCCTGACAGCACCATGACAGTTTACAAATGCCATGGCAACATCAGGAAGTT  
ACCCGATATGTCCCAAAGGGGGAGGAATGAATAATCCACCCCTTGTTTTAGCAAATAAGCAAGAAATAACCATA  
AAAGTGGGCAACCAGCAGCTCTAGGCGCTGCTCTTGTCCTATGGAGTAGCCTTCTTTTTTCTTTAACTTTCTT  
GATAAACTTGCTTTTACCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

## SEQ ID NO:4

30 MLLPLLLLSSLLGGSQAMDGRFWIRVQESVMVPEGLCISVPCSFYSYPRQDWTGSTPAYGYWFKAVTETTKGAPVA  
TNHQREVEEMSTRGRFQLTGDPKGNCSLVIRDAQMDESOYFFRVERGSYVRNFMNDGFFLKVTVLSTPRP  
QDHNTDLTCHVDFSRKGVSAQRTVRLRVAYAPRDLVISISRDNTPALEPQPQGNVPYLEAQKGQFLRLCAADS  
QPPATLSWVLQNRVLSSHPWGPRLGLELPGVKAGDSGRYTCRAENRLGSQORALDSVQYPPENLRVMVSQA  
NRTVLENLNGTSLPVLEGQSLCLVCVTHSSPPARLSWTQRGQVLSQSPDPGVLELPRVQVEHEGEFTCHAR  
35 HPLGSPARLSQPLRALLPEAAGPLLLLG\*

- 33 -

## SEQUENCE LISTING

## (1) GENERAL INFORMATION

5

(i) APPLICANT: SmithKline Beecham Corporation

10

(ii) TITLE OF THE INVENTION: Sialoadhesin Family 4 cDNA

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:

15

(A) ADDRESSEE: Ratner &amp; Prestia

(B) STREET: P.O. Box 980

(C) CITY: Valley Forge

(D) STATE: PA

(E) COUNTRY: USA

(F) ZIP: 19482

20

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: DOS

25

(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: TO BE ASSIGNED

(B) FILING DATE: 26-MAY-1998

30

(C) CLASSIFICATION: UNKNOWN

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 60/047,572

(B) FILING DATE: 27-MAY-1997

35

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Prestia, Paul F

40

(B) REGISTRATION NUMBER: 23,031

(C) REFERENCE/DOCKET NUMBER: GH-70026

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 610-407-0700

45

(B) TELEFAX: 610-407-0701

(C) TELEX:

## (2) INFORMATION FOR SEQ ID NO:1:

50

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3099 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

55

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- 34 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

	TCCGAGAGAA	CAGGCCTGTC	TCAGGCAGGC	CCTGCGCCTC	CTATGCGGAG	ATGCTACTGC	60
5	CACTGCTGCT	GTCCCTCGCTG	CTGGGCGGGT	CCCAGGCTAT	GGATGGGAGA	TTCTGGATAC	120
	GAGTGCAGGA	GTCAGTGATG	GTGCCGGAGG	GCCTGTGCAT	CTCTGTGCCC	TGCTCTTTCT	180
	CCTACCCCCG	ACAAGACTGG	ACAGGGTCTA	CCCCAGCTTA	TGGCTACTGG	TTCAAAGCAG	240
	TGACTGAGAC	AACCAAGGGT	GCTCCTGTGG	CCACAAACCA	CCAGAGTCGA	GAGGTGGAAA	300
	TGAGCACCCG	GGGCCGATTG	CAGCTCACTG	GGGATCCCGC	CAAGGGGAAC	TGCTCCTTGG	360
10	TGATCAGAGA	CGCGCAGATG	CAGGATGAGT	CACAGTACTT	CTTTCGGGTG	GAGAGAGGAA	420
	GCTATGTGAG	ATATAATTTC	ATGAACGATG	GGTTCTTTCT	AAAAGTAACA	GTGCTCAGCT	480
	TCACGCCCAG	ACCCACAGGAC	CACAACACCG	ACCTCACCTG	CCATGTGGAC	TTCTCCAGAA	540
	AGGGTGTGAG	CGCACAGAGG	ACCGTCCGAC	TCCGTGTGGC	CTATGCCCCC	AGAGACCTTG	600
	TTATCACTGAT	TTCACTGTGAC	AACACGCCAG	CCCTGGAGCC	CCAGCCCCAG	GGAAATGTCC	660
15	CATACCTGGA	AGCCCCAAAA	GGCCAGTTCC	TGCGGCTCCT	CTGTGCTGCT	GACAGCCAGC	720
	CCCCTGCCAC	ACTGAGCTGG	GTCCTGCAGA	ACAGAGTCCT	CTCCTCGTCC	CATCCCTGGG	780
	GCCCTAGACC	CCTGGGGCTG	GAGCTGCCCC	GGGTGAAGGC	TGGGGATTCA	GGGCGCTACA	840
	CCTGCCGAGC	GGAGAACAGG	CTTGGCTCCC	AGCAGCGAGC	CCTGGACCTC	TCTGTGCAGT	900
	ATCCTCCAGA	GAACCTGAGA	GTGATGGTTT	CCCAAGCAAA	CAGGACAGTC	CTGGAAAACC	960
20	TTGGGAACGG	CACGTCTCTC	CCAGTACTGG	AGGGCCAAAG	CCTGTGCCTG	GTCTGTGTCA	1020
	CACACAGCAG	CCCCCAGGCC	AGGCTGAGCT	GGACCCAGAG	GGGACAGGTT	CTGAGCCCCT	1080
	CCCAGCCCTC	AGACCCCGGG	GTCCTGGAGC	TGCCTCGGGT	TCAAGTGGAG	CACGAAGGAG	1140
	AGTTCACCTG	CCACGCTCGG	CACCCACTGG	GCTCCCAGCA	CGTCTCTCTC	AGCCTCTCCG	1200
	TGCACTACTC	CCCGAAGCTG	CTGGGCCCCC	CCTGCTCCTG	GGAGGCTGAG	GGTCTGCACT	1260
25	GCAGCTGCTC	CTCCCAGGCC	AGCCCGGCC	CCTCTCTGCG	CTGGTGGCTT	GGGGAGGAGC	1320
	TGCTGGAGGG	GAACAGCAGC	CAGGACTCCT	TCGAGGTCAC	CCCCAGCTCA	GCCGGGCCCT	1380
	GGGCCAACAG	CTCCCTGAGC	TCCATGGAG	GGCTCAGCTC	CGGCCTCAGG	TCCGCTGTG	1440
	AGGCCTGGAA	CGTCCATGGG	GCCCAGAGTG	GATCCATCCT	GCAGCTGCCA	GATAAGAAGG	1500
	GACTCATCTC	AACGGCATTC	TCCAACGGAG	CGTTTCTGGG	AATCGGCATC	ACGGCTCTTC	1560
30	TTTTCTCTG	CCTGGCCCTG	ATCATCATGA	AGATTCTACC	GAAGAGACGG	ACTCAGACAG	1620
	AAACCCCGAG	GCCCAGGTTT	TCCCGGCACA	GCACGATCCT	GGATTACATC	AATGTGGTCC	1680
	CGACGGCTGG	CCCCCTGGCT	CAGAAGCGGA	ATCAGAAAGC	CACACCAAAC	AGTCCTCGGA	1740
	CCCCTCTTCC	ACCAGGTGCT	CCCTCCCCAG	AATCAAAGAA	GAACCAGAAA	AAGCAGTATC	1800
	AGTTGCCCAG	TTTCCAGAAA	CCCAAATCAT	CCACTCAAGC	CCCAGAATCC	CAGGAGAGCC	1860
35	AAGAGGAGCT	CCATTATGCC	ACGCTCAACT	TCCCAGGCGT	CAGACCCAGG	CCTGAGGCCC	1920
	GGATGCCCAA	GGGCACCCAG	GCGGATTATG	CAGAAGTCAA	GTTCCAATGA	GGGTCTCTTA	1980
	GGCTTTAGGA	CTGGGACTTC	GGCTAGGGAG	GAAGGTAGAG	TAAGAGGTTG	AAGATAACAG	2040
	AGTGCAAAGT	TTCTTTCTCT	CCCTCTCTCT	CTCTCTTTCT	CTCTCTCTCT	CTCTTTCTCT	2100
	CTCTTTAAAA	AACATCTGGC	CAGGCAGCAG	AGTGGCTCAC	GCCTGTAATC	CCAGCACTTT	2160
40	GGGAGGTTGA	GGTGGGCAGA	TGCGCTGAGG	TGCGGAGTTC	GAGACCAGCC	TGGCCAACCT	2220
	GGTGAAACCC	CGTCTCTACT	AAAAATACAA	AAATTAGCTG	GGCATGGTGG	CAGGCGCCTG	2280
	TAATCCTACC	TACTTGGAAG	GCTGAGGCAG	GAGAATCACT	TGAACCTGGG	AGACGGAGGT	2340
	TGCAGTGAGC	CAAGATCACA	CCATTGCACG	CCAGCCTGGG	CAACAAAGCG	AGACTCCATC	2400
	TCAAAAAAAA	AATCCTCCAA	ATGGGTTGGG	TGTCTGTAAT	CCCAGCACTT	TGGGAGGCTA	2460
45	AGGTGGGTGG	ATTGCTTGAG	CCCAGGAGTT	CGAGACCAGC	CTGGGCAACA	TGGTGAAACC	2520
	CCATCTCTAC	AAAAAATACA	AAACATAGCT	GGGCTTGGTG	GTGTGTGCCT	GTAAGTCCCC	2580
	AGCTGTCAGA	CATTTAAACC	AGAGCAACTC	CATCXTGGAA	TAGGAGGCTG	AATAAAATGA	2640
	GGCTGAGACC	TXACTGGGCT	GCATTCTCCA	GACAGTGGAG	GCATTCTAAG	TCACAGGATG	2700
	AGACAGGAGG	TCCCGTACAA	GATACAGGTC	ATAAAGACTT	TGCTGATAAA	ACAGATTGCA	2760
50	GTAAAGAAGC	CAACCAAATC	CCACCAAAC	CAAGTTGGCC	ACGAGAGTGA	CCTCTGGTCG	2820
	TCCTCACTGC	TACACTCCTG	ACAGCACCAT	GACAGTTTAC	AAATGCCATG	GCAACATCAG	2880
	GAAGTTACCC	GATATGTCCC	AAAAGGGGCA	GGAATGAATA	ATCCACCCCT	TGTTTAGCAA	2940
	ATAAGCAAGA	AATAACCATA	AAAGTGGGCA	ACCAGCAGCT	CTAGGCGCTG	CTCTTGTCTT	3000
	ATGGAGTAGC	CTTCTTTTTT	CCTTTAACTT	TCTTGATAAA	CTTGCTTTCA	CCTAAAAAAA	3060
55	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA			3099

- 35 -

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 639 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Leu Leu Pro Leu Leu Ser Ser Leu Leu Gly Gly Ser Gln Ala
 1           5           10           15
Met Asp Gly Arg Phe Trp Ile Arg Val Gln Glu Ser Val Met Val Pro
15           20           25           30
Glu Gly Leu Cys Ile Ser Val Pro Cys Ser Phe Ser Tyr Pro Arg Gln
           35           40           45
Asp Trp Thr Gly Ser Thr Pro Ala Tyr Gly Tyr Trp Phe Lys Ala Val
           50           55           60
20 Thr Glu Thr Thr Lys Gly Ala Pro Val Ala Thr Asn His Gln Ser Arg
           65           70           75           80
Glu Val Glu Met Ser Thr Arg Gly Arg Phe Gln Leu Thr Gly Asp Pro
           85           90           95
Ala Lys Gly Asn Cys Ser Leu Val Ile Arg Asp Ala Gln Met Gln Asp
25           100          105          110
Glu Ser Gln Tyr Phe Phe Arg Val Glu Arg Gly Ser Tyr Val Arg Tyr
           115          120          125
Asn Phe Met Asn Asp Gly Phe Phe Leu Lys Val Thr Val Leu Ser Phe
           130          135          140
30 Thr Pro Arg Pro Gln Asp His Asn Thr Asp Leu Thr Cys His Val Asp
           145          150          155          160
Phe Ser Arg Lys Gly Val Ser Ala Gln Arg Thr Val Arg Leu Arg Val
           165          170          175
Ala Tyr Ala Pro Arg Asp Leu Val Ile Ser Ile Ser Arg Asp Asn Thr
35           180          185          190
Pro Ala Leu Glu Pro Gln Pro Gln Gly Asn Val Pro Tyr Leu Glu Ala
           195          200          205
Gln Lys Gly Gln Phe Leu Arg Leu Leu Cys Ala Ala Asp Ser Gln Pro
           210          215          220
40 Pro Ala Thr Leu Ser Trp Val Leu Gln Asn Arg Val Leu Ser Ser Ser
           225          230          235          240
His Pro Trp Gly Pro Arg Pro Leu Gly Leu Glu Leu Pro Gly Val Lys
           245          250          255
Ala Gly Asp Ser Gly Arg Tyr Thr Cys Arg Ala Glu Asn Arg Leu Gly
45           260          265          270
Ser Gln Gln Arg Ala Leu Asp Leu Ser Val Gln Tyr Pro Pro Glu Asn
           275          280          285
Leu Arg Val Met Val Ser Gln Ala Asn Arg Thr Val Leu Glu Asn Leu
           290          295          300
50 Gly Asn Gly Thr Ser Leu Pro Val Leu Glu Gly Gln Ser Leu Cys Leu
           305          310          315          320
Val Cys Val Thr His Ser Ser Pro Pro Ala Arg Leu Ser Trp Thr Gln
           325          330          335
Arg Gly Gln Val Leu Ser Pro Ser Gln Pro Ser Asp Pro Gly Val Leu
55           340          345          350
Glu Leu Pro Arg Val Gln Val Glu His Glu Gly Glu Phe Thr Cys His

```

[illegible]

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3099 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

BNSDOCID: &lt;WO 9853840A1 | &gt;

- 37 -

```

AGGGTGTGAG CGCACAGAGG ACCGTCCGAC TCCGTGTGGC CTATGCCCCC AGAGACCTTG      600
TTATCAGCAT TTCACGTGAC AACACGCCAG CCCTGGAGCC CCAGCCCCAG GGAAATGTCC      660
CATACCTGGA AGCCCCAAAA GGCCAGTTCC TGC GGCTCCT CTGTGCTGCT GACAGCCAGC      720
CCCCTGCCAC ACTGAGCTGG GTCCTGCAGA ACAGAGTCCCT CTCCTCGTCC CATCCCTGGG      780
5  GCCCTAGACC CCTGGGGCTG GAGCTGCCCC GGGTGAAGGC TGGGGATTCA GGGCGCTACA      840
CCTGCCGAGC GGAGAACAGG CTTGGCTCCC AGCAGCGAGC CCTGGACCTC TCTGTGCAGT      900
ATCCTCCAGA GAACCTGAGA GTGATGGTTT CCCAAGCAAA CAGGACAGTC CTGGA AAAACC      960
TTGGGAACGG CACGTCTCTC CCAGTACTGG AGGGCCAAAAG CCTGTGCCTG GTCTGTGTCA     1020
CACACAGCAG CCCCCAGCC AGGCTGAGCT GGACCCAGAG GGGACAGGTT CTGAGCCCCT     1080
10  CCCAGCCCTC AGACCCCGGG GTCCTGGAGC TGCCTCGGGT TCAAGTGGAG CACGAAGGAG     1140
AGTTCACCTG CCACGCTCGG CACCCACTGG GCTCCAGCA CGTCTCTCTC AGCCTCTCCG     1200
TGCACTACTC CCCGAAGCTG CTGGGCCCTT CCTGCTCCTG GGAGGCTGAG GGTCTGCACT     1260
GCAGCTGCTC CTCCAGGCC AGCCCGGCC CCTCTCTGCG CTGGTGGCTT GCGGAGGAGC     1320
TGCTGGAGGG GAACAGCAGC CAGGACTCCT TCGAGGTCAC CCCAGCTCA GCGGGGCCCT     1380
15  GGGCCAAACAG CTCCCTGAGC CTCCATGGAG GGCTCAGCTC CGGCCTCAGG CTCCGCTGTG     1440
AGGCCTGGAA CGTCCATGGG GCCCAGAGTG GATCCATCCT GCAGCTGCCA GATAAGAAGG     1500
GACTCATCTC AACGGCATTC TCCAACGGAG CGTTTCTGGG AATCGGCATC ACGGCTCTTC     1560
TTTTCTCTG CCTGGCCCTG ATCATCATGA AGATTCTACC GAAGAGACGG ACTCAGACAG     1620
AAACCCCGAG GCCCAGGTTT TCCCGGCACA GCACGATCCT GGATTACATC AATGTGGTCC     1680
20  CGACGGCTGG CCCCCTGGCT CAGAAGCGGA ATCAGAAAGC CACACCAAAC AGTCCTCGGA     1740
CCCCTCTTCC ACCAGGTGCT CCCTCCCCAG AATCAAAGAA GAACCAGAAA AAGCAGTATC     1800
AGTTGCCCCAG TTTCCAGAA CCCAAATCAT CCACTCAAGC CCCAGAATCC CAGGAGAGCC     1860
AAGAGGAGCT CCATTATGCC ACGCTCAACT TCCAGGCGT CAGACCCAGG CCTGAGGCCC     1920
GGATGCCCAA GGGCAGCCAG GCGGATTATG CAGAAGTCAA GTTCCAATGA GGGTCTCTTA     1980
25  GGCTTTAGGA CTGGGACTTC GGCTAGGGAG GAAGGTAGAG TAAGAGGTTG AAGATAACAG     2040
AGTGCAAAGT TTCTTCTCTT CCCTCTCTCT CTCTCTTCTT CTCTCTCTCT CTCTTTCTCT     2100
CTCTTTAAAA AACATCTGGC CAGGCAGCAC AGTGGCTCAC GCCTGTAATC CCAGCACTTT     2160
GGGAGGTTGA GGTGGGCAGA TCGCCTGAGG TCGGGAGTTC GAGACCAGCC TGGCCAACTT     2220
GGTGAAACCC CGTCTCTACT AAAAATACAA AAATTAGCTG GGCATGGTGG CAGGCGCCTG     2280
30  TAATCCTACC TACTTGGGAA GCTGAGGCAG GAGAATCACT TGAACCTGGG AGACGGAGGT     2340
TGCAGTGAGC CAAGATCACA CCATTGCACG CCAGCCTGGG CAACAAAGCG AGACTCCATC     2400
TCAAAAAAAA AATCCTCCAA ATGGGTGGG TGTCTGTAAT CCCAGCACTT TGGGAGGCTA     2460
AGGTGGGTGG AFTGCTTGAG CCCAGGAGTT CGAGACCAGC CTGGGCAACA TGGTGAAACC     2520
CCATCTCTAC AAAAAATACA AAACATAGCT GGGCTTGGTG GTGTGTGCCT GTAAGTCCCC     2580
35  AGCTGTCAGA CATTAAACC AGAGCAACTC CATCXTGGAA TAGGAGGCTG AATAAAATGA     2640
GGCTGAGACC TXACTGGGCT GCATTCTCCA GACAGTGGAG GCATTCTAAG TCACAGGATG     2700
AGACAGGAGG TCCCGTACAA GATACAGGTC ATAAAGACTT TGCTGATAAA ACAGATTGCA     2760
GTAAAGAAGC CAACCAAATC CCACCAAAC CAAGTTGGCC ACGAGAGTGA CCTCTGGTCG     2820
TCCTCACTGC TACACTCCTG ACAGCACCAT GACAGTTTAC AAATGCCATG GCAACATCAG     2880
40  GAAGTTACCC GATATGTCCC AAAAGGGGGA GGAATGAATA ATCCACCCCT TGTTTAGCAA     2940
ATAAGCAAGA AATAACCATA AAAGTGGGCA ACCAGCAGCT CTAGGCGCTG CTCTTGTCTT     3000
ATGGAGTAGC CTTCTTTTTT CCTTTAACTT TCTTGATAAA CTTGCTTTCA CCTAAAAAAA     3060
AAAAAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA AAAA     3099

```

45 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 399 amino acids

(B) TYPE: amino acid

50 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

55

Met Leu Leu Pro Leu Leu Leu Ser Ser Leu Leu Gly Gly Ser Gln Ala

- 38 -

	1		5		10		15									
	Met	Asp	Gly	Arg	Phe	Trp	Ile	Arg	Val	Gln	Glu	Ser	Val	Met	Val	Pro
			20						25					30		
5	Glu	Gly	Leu	Cys	Ile	Ser	Val	Pro	Cys	Ser	Phe	Ser	Tyr	Pro	Arg	Gln
			35					40					45			
	Asp	Trp	Thr	Gly	Ser	Thr	Pro	Ala	Tyr	Gly	Tyr	Trp	Phe	Lys	Ala	Val
		50					55					60				
	Thr	Glu	Thr	Thr	Lys	Gly	Ala	Pro	Val	Ala	Thr	Asn	His	Gln	Ser	Arg
	65					70					75				80	
10	Glu	Val	Glu	Met	Ser	Thr	Arg	Gly	Arg	Phe	Gln	Leu	Thr	Gly	Asp	Pro
				85					90					95		
	Ala	Lys	Gly	Asn	Cys	Ser	Leu	Val	Ile	Arg	Asp	Ala	Gln	Met	Gln	Asp
			100						105				110			
	Glu	Ser	Gln	Tyr	Phe	Phe	Arg	Val	Glu	Arg	Gly	Ser	Tyr	Val	Arg	Tyr
15			115					120					125			
	Asn	Phe	Met	Asn	Asp	Gly	Phe	Phe	Leu	Lys	Val	Thr	Val	Leu	Ser	Phe
		130				135						140				
	Thr	Pro	Arg	Pro	Gln	Asp	His	Asn	Thr	Asp	Leu	Thr	Cys	His	Val	Asp
	145				150						155				160	
20	Phe	Ser	Arg	Lys	Gly	Val	Ser	Ala	Gln	Arg	Thr	Val	Arg	Leu	Arg	Val
				165					170					175		
	Ala	Tyr	Ala	Pro	Arg	Asp	Leu	Val	Ile	Ser	Ile	Ser	Arg	Asp	Asn	Thr
			180					185					190			
	Pro	Ala	Leu	Glu	Pro	Gln	Pro	Gln	Gly	Asn	Val	Pro	Tyr	Leu	Glu	Ala
25			195				200					205				
	Gln	Lys	Gly	Gln	Phe	Leu	Arg	Leu	Leu	Cys	Ala	Ala	Asp	Ser	Gln	Pro
		210				215						220				
	Pro	Ala	Thr	Leu	Ser	Trp	Val	Leu	Gln	Asn	Arg	Val	Leu	Ser	Ser	Ser
	225				230						235				240	
30	His	Pro	Trp	Gly	Pro	Arg	Pro	Leu	Gly	Leu	Glu	Leu	Pro	Gly	Val	Lys
				245					250					255		
	Ala	Gly	Asp	Ser	Gly	Arg	Tyr	Thr	Cys	Arg	Ala	Glu	Asn	Arg	Leu	Gly
			260					265					270			
	Ser	Gln	Gln	Arg	Ala	Leu	Asp	Leu	Ser	Val	Gln	Tyr	Pro	Pro	Glu	Asn
35			275				280					285				
	Leu	Arg	Val	Met	Val	Ser	Gln	Ala	Asn	Arg	Thr	Val	Leu	Glu	Asn	Leu
		290				295					300					
	Gly	Asn	Gly	Thr	Ser	Leu	Pro	Val	Leu	Glu	Gly	Gln	Ser	Leu	Cys	Leu
	305				310					315					320	
40	Val	Cys	Val	Thr	His	Ser	Ser	Pro	Pro	Ala	Arg	Leu	Ser	Trp	Thr	Gln
				325					330					335		
	Arg	Gly	Gln	Val	Leu	Ser	Pro	Ser	Gln	Pro	Ser	Asp	Pro	Gly	Val	Leu
			340					345					350			
	Glu	Leu	Pro	Arg	Val	Gln	Val	Glu	His	Glu	Gly	Glu	Phe	Thr	Cys	His
45			355				360					365				
	Ala	Arg	His	Pro	Leu	Gly	Ser	Pro	Ala	Arg	Leu	Ser	Gln	Pro	Leu	Arg
		370				375					380					
	Ala	Leu	Leu	Pro	Glu	Ala	Ala	Gly	Pro	Leu	Leu	Leu	Leu	Gly	Gly	
	385				390					395						



- 39 -

**What is claimed is:**

**1. An isolated polypeptide selected from the group consisting of:**

(i) an isolated polypeptide comprising an amino acid sequence selected from the group having at least:

- (a) 70% identity;
- (b) 80% identity;
- (c) 90% identity; or
- (d) 95% identity

to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2;

(ii) an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2  
or

(iii) an isolated polypeptide which is the amino acid sequence of SEQ ID NO:2.

**2. An isolated polynucleotide selected from the group consisting of:**

(i) an isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least

- (a) 70% identity;
- (b) 80% identity;
- (c) 90% identity; or
- (d) 95% identity;

to the amino acid sequence of SEQ ID NO:2, over the entire length of SEQ ID NO:2;

(ii) an isolated polynucleotide comprising a nucleotide sequence that has at least:

- (a) 70% identity

- 40 -

- (b) 80 % identity;
- (c) 90 % identity; or
- (d) 95 % identity;

over its entire length to a nucleotide sequence encoding the polypeptide of SEQ ID NO:2;

(iii) an isolated polynucleotide comprising a nucleotide sequence which has at least:

- (a) 70 % identity;
- (b) 80 % identity;
- (c) 90 % identity; or
- (d) 95 % identity;

to that of SEQ ID NO: 1 over the entire length of SEQ ID NO:1;

(iv) an isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2;

(vi) an isolated polynucleotide which is the polynucleotide of SEQ ID NO: 1; or

(vi) an isolated polynucleotide obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO: 1 or a fragment thereof.;

or a nucleotide sequence complementary to said isolated polynucleotide.

3. An antibody immunospecific for the polypeptide of claim 1.

4. A method for the treatment of a subject:

(i) in need of enhanced activity or expression of the polypeptide of claim 1 comprising:

(a) administering to the subject a therapeutically effective amount of an agonist to said polypeptide; and/or

- 41 -

(b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence encoding said polypeptide in a form so as to effect production of said polypeptide activity *in vivo.*; or

(ii) having need to inhibit activity or expression of the polypeptide of claim 1 comprising:

(a) administering to the subject a therapeutically effective amount of an antagonist to said polypeptide; and/or

(b) administering to the subject a nucleic acid molecule that inhibits the expression of a nucleotide sequence encoding said polypeptide;

and/or

(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said polypeptide for its ligand, substrate , or receptor.

5. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of the polypeptide of claim 1 in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said polypeptide in the genome of said subject; and/or

(b) analyzing for the presence or amount of said polypeptide expression in a sample derived from said subject.

6. A method for screening to identify compounds which stimulate or which inhibit the function of the polypeptide of claim 1 which comprises a method selected from the group consisting of:

(a) measuring the binding of a candidate compound to the polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound;

- 42 -

- (b) measuring the binding of a candidate compound to the polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof in the presence of a labeled competitor;
- (c) testing whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells or cell membranes bearing the polypeptide;
- (d) mixing a candidate compound with a solution containing a polypeptide of claim 1, to form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a standard; or
- (e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide and said polypeptide in cells, using for instance, an ELISA assay.

- 7. An agonist or an antagonist of the polypeptide of claim 1.
- 8. An expression system comprising a polynucleotide capable of producing a polypeptide of claim 1 when said expression system is present in a compatible host cell.
- 9. A process for producing a recombinant host cell comprising transforming or transfecting a cell with the expression system of claim 8 such that the host cell, under appropriate culture conditions, produces a polypeptide comprising an amino acid sequence having at least 70 % identity to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2.
- 10. A recombinant host cell produced by the process of claim 9.

- 43 -

11. A membrane of a recombinant host cell of claim 10 expressing a polypeptide comprising an amino acid sequence having at least 70% identity to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2.

12. A process for producing a polypeptide comprising culturing a host cell of claim 10 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.

13. An isolated polynucleotide selected from the group consisting of:

- (a) an isolated polynucleotide comprising a nucleotide sequence which has at least 70%, 80%, 90%, 95%, 97% identity to SEQ ID NO:3 over the entire length of SEQ ID NO:3;
- (b) an isolated polynucleotide comprising the polynucleotide of SEQ ID NO:3;
- (c) the polynucleotide of SEQ ID NO:3; or
- (d) an isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide which has at least 70%, 80%, 90%, 95%, 97-99% identity to the amino acid sequence of SEQ ID NO:4, over the entire length of SEQ ID NO:4.

14. A polypeptide selected from the group consisting of:

- (a) a polypeptide which comprises an amino acid sequence which has at least 70%, 80%, 90%, 95%, 97-99% identity to that of SEQ ID NO:4 over the entire length of SEQ ID NO:4;
- (b) a polypeptide which has an amino acid sequence which is at least 70%, 80%, 90%, 95%, 97-99% identity to the amino acid sequence of SEQ ID NO:4 over the entire length of SEQ ID NO:4;
- (c) a polypeptide which comprises the amino acid of SEQ ID NO:4;
- (d) a polypeptide which is the polypeptide of SEQ ID NO:4;

- 44 -

(e) a polypeptide which is encoded by a polynucleotide comprising the sequence contained in SEQ ID NO:3.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/10791**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 143.1; 435/69.1, 71.1, 440, 455, 471; 514/8, 44; 530/350, 387.1, 388.2, 388.22; 536/23.1, 24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, BIOSIS, CA, EMBASE, MEDLINE, WPI  
search terms: sialoadhesin, saf-4, cd33, SEQ ID NOS. 1-4**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CROCKER et al. Sialoadhesin, a macrophage sialic acid binding receptor for haemopoietic cells with 17 immunoglobulin-like domains. EMBO J. 1994, Vol. 13, No. 19, pages 4490-4503, see entire document.	1-14
Y	MUCKLOW et al. Sialoadhesin (Sn) maps to mouse chromosome 2 and human chromosome 20 and is not linked to the other members of the sialoadhesin family, CD22, MAG and CD33. Genomics. 1995, Vol. 28, pages 344-346, see entire document.	1-14
Y	FREEMAN et al. Characterization of CD33 as a new member of the sialoadhesin family of cellular interaction molecules, Blood. 15 April 1995, Vol. 85, No. 8, pages 2005-2012, see entire document.	1-14



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

31 AUGUST 1998

Date of mailing of the international search report

30 SEP 1998

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

*Phillip Gambel*  
PHILLIP GAMBEL

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/10791

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A61K 38/00, 39/395, 48/00; C07H 21/04; C07K 14/435, 14/705, 16/00, 16/18, 16/28; C12N 15/01, 15/11, 15/12, 15/63

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/130.1, 143.1; 435/69.1, 71.1, 440, 455, 471; 514/8, 44; 530/350, 387.1, 388.2, 388.22; 536/23.1, 24.5